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*SYMPOSIUM ON THREE-DIMENSIONAL STRUCTURE OF MACROMOLECULES OF BIOLOGICAL ORIGIN**

BY INVITATION OF THE COMMITTEE ON ARRANGEMENTS FOR THE AUTUMN MEETING

Presented before the Academy on October 19, 1966

Chairman, WALTER KAUZMANN

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* *Editorial Note:* In the program as presented at the Autumn Meeting of the Academy in Durham, North Carolina, October 17–19, 1966, the authors and titles read as follows:

DAVID C. PHILLIPS, Oxford University, England: *The Hen Egg-White Lysozyme Molecule.*

JOHN A. RUPLEY, University of Arizona, Tucson, Arizona: *Some Relationships between the Structure and the Enzymic Properties of Lysozyme.*

WILLIAM N. LIPSCOMB, Harvard University, Cambridge, Massachusetts: *Recent Results on the Structure of Carboxypeptidase A.*

MICHAEL G. ROSSMANN, Purdue University, Lafayette, Indiana: *The Crystal Structure of Lactic Dehydrogenase.*

FREDERIC M. RICHARDS, Yale University, New Haven, Connecticut: *Some Properties of Enzymes in the Solid State.*

In the published papers which follow, the names of several co-authors have been added to some of the papers and some of the titles have been altered in order to designate more specifically the character of the material presented.

THE HEN EGG-WHITE LYSOZYME MOLECULE

By David C. Phillips

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1. *Introduction.*—The three-dimensional structure of the hen egg-white lysozyme molecule has been determined by X-ray analysis of the tetragonal crystals grown at pH 4.7 from 1 *M* sodium chloride solution. The crystals have unit cell dimensions $a = b = 79.1$, $c = 37.9$ Å and space group $P4_32_12$, and there are eight molecules in the unit cell, one per asymmetric unit (Palmer *et. al.*, 1948; Blake *et. al.*, 1962). Liquid of crystallization constitutes about 33.5 per cent of the weight of the crystal (Steinrauf, 1959). The phases of the X-ray reflections were determined by the method of multiple isomorphous replacement developed to take systematic account of anomalous scattering effects (North, 1965). Isomorphous crystals of three different heavy atom derivatives were used, incorporating, respectively, *ortho*-mercuri hydroxytoluene *para*-sulfonic acid, $UO_2F_5^{\equiv}$ (Holmes and Leberman, 1963), and an ion derived from $UO_2(NO_3)_2$. The best electron density distribution (Blow and Crick, 1959) was calculated on the London University Atlas computer from all reflections with interplanar spacings greater than 2 Å. It has been interpreted with reference to the known amino acid sequence of lysozyme as determined independently by Jollès and Canfield and their colleagues (Jollès and Jollès, 1963, 1964; Canfield, 1963; Canfield and Liu, 1965). Preliminary accounts of the work have been published (Blake *et. al.*, 1965, 1967) and a full account is in course of preparation.

2. *Interpretation of the Electron Density Map.*—Hen egg-white lysozyme has a molecular weight of about 14,600 and each molecule comprises 129 amino acid residues. The primary structure is shown in Figure 1. The problem of interpreting the electron density map in terms of this chemical structure can be assessed by examination of the part of the map shown in Figure 2. This figure shows 10 of the 60 parallel sections that make up the whole map, and it includes the features which were first identified. The individual atoms are not resolved but the characteristic shapes of various groups of atoms can be recognized without too much difficulty. Thus the region of highest electron density, at lower right of center, was found to correspond to the half-cysteine residue 30 since it clearly forms part of a helical conformation of the polypeptide chain, the axis of which is marked *HH'*, and is four residues removed from an identifiable phenylalanine residue (34) nearer the carboxyl end of the chain. The course of the main chain was easy to follow for all but a few residues, to a great extent because the corresponding ribbon of electron density exhibited regularly spaced promontories characteristic of the carbonyl groups, some of which can be seen clearly in Figure 2. Many of the side chains were also identifiable; some of them are marked in the figure, but no attempt was made to interpret the map without recourse to the known amino acid sequence, knowledge of which was of great benefit.

As a result of systematic study of the map and precise model building, the positions of some 95 per cent of the atoms in the molecule have been determined to within about 0.25 Å. There is appropriate electron density for 114 of the 129

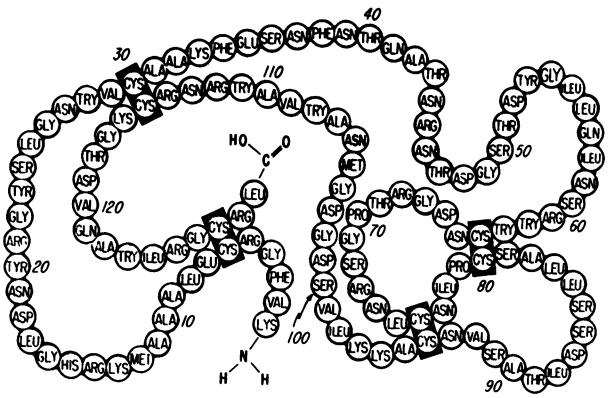


FIG. 1.—Amino acid sequence of hen egg-white lysozyme reproduced from Canfield and Liu, 1965.

side chains and those that are fitted with difficulty are all on the surface of the molecule where they may take up alternative conformations. It must be clearly understood, however, before any attempt is made to describe the molecule or to draw conclusions from its apparent conformation, that there is often some freedom allowed in fitting the model to the electron density map. The proposed conformations of the main chain, and more particularly the side chains, are to some extent, therefore, biased towards those expected and they must be interpreted with caution at this stage of the analysis.

3. *Description of the Structure.—Conformation of the main chain:* On the reasonable assumption that the dimensions of the peptide group are constant throughout the course of the main chain, its conformation can be described most economically in terms of the two dihedral angles ϕ and ψ, corresponding to rotations about the NCα and CαC' bonds (Edsall *et al.*, 1966). Preliminary values of these angles are listed in Table 1 and, although their accuracy cannot be assessed reliably at this stage, inspection of the table reveals some general features of the molecule that deserve comment. Thus, for example, the α-helix content is much lower than that observed in myoglobin, though it is not clear how it should be assessed. If the values of ϕ,ψ for the α-helix are taken to be 113°, 136° (Arnott and Wonacott, 1966), then for 62 residues both of these angles are within 40° of the ideal value. A number of these are isolated residues in the α-helical conformation, however, and only 46 of them are included in continuous sequences of 4 or more that contribute to recognizable helices. Furthermore, some of these helices are markedly irregular and there appears to be a tendency for the main chain carbonyl groups in them to point away from the helix axes, so that the α-helical hydrogen-bonding scheme is perturbed. These features are the subject of continuing analysis.

Residues 41–54 provide an example of another standard conformation which has not been found previously in a globular protein. This part of the chain doubles back on itself, as shown in Figure 3, to form a section of antiparallel pleated sheet (Pauling and Corey, 1951). The rest of the main chain gives the impression of being irregularly folded, and is not easily described in a short space.

The side chains: Despite the freedom allowed in interpretation of the electron density map, as has been stressed above, some conformational differences can be

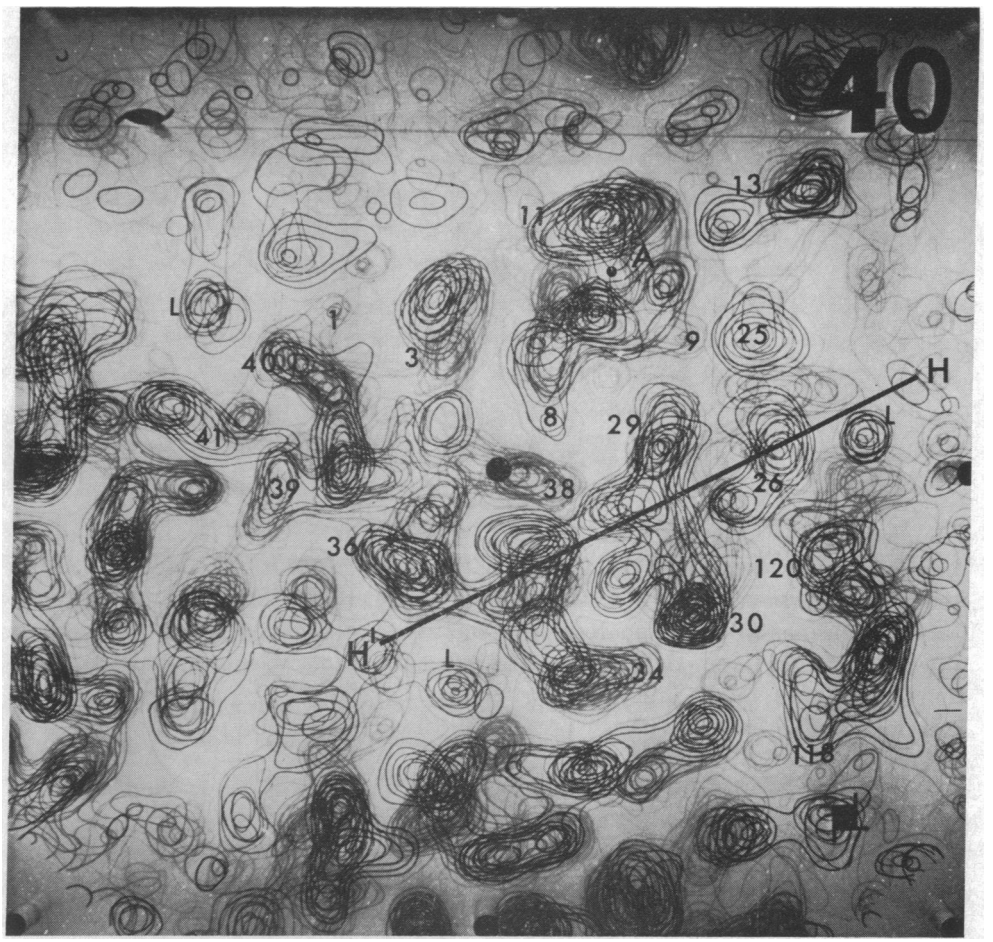


FIG. 2.—The sections $Z = 40$–$49/60$ of the 2-Å electron density map. Contours at intervals of 0.25 eA^{-3}. HH' shows the course of a length of helix lying in the plane of the sections. A indicates a length of helix more nearly normal to the sections. The densities corresponding to a number of amino acid side chains are numbered in accordance with Fig. 1 and three probable Cl^- ions are labeled L.

distinguished in the side chains. Thus, there are examples of each of the three staggered conformations that arise from rotation about the $\alpha - \beta$ bond of valine. Detailed analysis of the side chain conformations is still in progress, but the disulphide bridges deserve special mention here. Two conformations are found which are mirror images of each other and there are two examples of each type. The links between residues 64 and 80, 76 and 94 can be regarded as right-handed while those between 6 and 127, 30 and 115 are left-handed. All of the dihedral angles about the S–S bond are within the range $100 \pm 5°$.

The general distribution of side chains conforms closely to the pattern observed in myoglobin in that all of the ionizable groups and most of the polar side chains are distributed over the surface of the molecule, while the majority of the nonpolar side chains lie within its interior. There are a number of hydrophobic groups on the surface, however, and they include most prominently Trp 62, Trp 63, Ile 98, Val

TABLE 1

DIHEDRAL ANGLES

Residue	ϕ	ψ	Residue	ϕ	ψ	Residue	ϕ	ψ
2	56.0	301.4	45	76.4	315.2	87	51.9	312.3
3	106.9	337.9	46	90.1	2.8	88	110.5	176.7
4	78.9	336.0	47	111.3	161.3	89	144.2	122.0
5	132.7	114.5	48	86.9	216.9	90	149.1	100.1
6	142.5	116.4	49	276.5	107.2	91	125.9	120.2
7	126.8	153.4	50	184.9	291.2	92	124.9	134.0
8	119.1	121.4	51	35.7	331.9	93	113.6	150.0
9	126.2	129.9	52	69.0	302.6	94	108.6	128.9
10	115.1	140.4	53	65.0	314.0	95	128.0	141.4
11	140.9	120.9	54	255.2	352.2	96	117.1	128.5
12	109.0	152.9	55	133.7	144.6	97	104.7	162.1
13	123.3	114.1	56	71.5	189.9	98	91.4	141.2
14	142.3	105.4	57	232.6	228.1	99	102.8	169.3
15	116.9	176.4	58	114.9	317.7	100	66.0	184.3
16	277.1	171.6	59	92.2	342.6	101	97.8	123.5
17	127.8	145.9	60	74.9	185.0	102	3.5	149.2
18	104.7	330.3	61	98.8	162.5	103	84.6	181.0
19	241.4	182.4	62	45.0	138.7	104	233.8	36.7
20	103.4	318.6	63	93.5	145.5	105	102.6	187.8
21	241.5	219.8	64	26.9	330.9	106	107.7	154.3
22	239.6	202.2	65	83.2	329.4	107	113.3	160.7
23	84.7	298.1	66	35.0	185.2	108	71.8	257.5
24	96.9	352.2	67	250.5	179.7	109	149.9	126.4
25	149.4	131.3	68	45.5	200.1	110	117.7	140.7
26	123.4	131.7	69	59.8	269.5	111	124.0	127.2
27	127.9	125.7	70	132.0	141.4	112	123.1	112.9
28	120.0	138.4	71	98.8	171.4	113	122.3	134.6
29	102.1	131.5	72	127.6	313.3	114	76.7	163.3
30	99.3	147.0	73	50.8	313.8	115	63.6	135.6
31	124.7	111.9	74	60.9	261.6	116	127.4	285.1
32	115.4	150.0	75	124.4	135.1	117	275.9	201.6
33	115.2	128.8	76	81.1	200.6	118	65.4	349.6
34	108.2	150.6	77	217.5	223.1	119	92.8	262.5
35	97.6	131.0	78	45.3	321.4	120	116.0	156.6
36	76.7	156.1	79	101.9	309.4	121	125.2	142.4
37	250.9	181.4	80	165.7	117.1	122	121.2	160.6
38	292.4	153.4	81	133.5	141.0	123	115.9	141.6
39	95.6	292.2	82	127.8	137.6	124	82.6	176.2
40	109.8	165.3	83	127.7	140.7	125	109.7	308.9
41	93.6	138.8	84	95.8	182.5	126	279 0	167.6
42	143.0	330.1	85	119.6	326.1	127	103.7	321.0
43	36.1	329.8	86	90.8	186.7	128	92 0	283.2
44	46.2	284.8						

109, and Trp 123. On the other hand, only Gln 57 and Ser 91 of the polar side chains appear to be shielded from contact with the surrounding liquid.

Water molecules and chloride ions: The electron density distribution is comparatively featureless over a considerable volume of the unit cell, indicating regions occupied by mother liquor. In some other regions, however, there are peaks of electron density strongly suggestive of ordered water molecules at hydrogen-bonding distances from each other or from appropriate groups on the protein surface. Some of these peaks are rather larger than the majority; three of them are marked *L* in Figure 2, and these are thought to represent Cl^- ions of which there are known to be about 16 associated with each lysozyme molecule in the crystal.

4. *The Folding of Lysozyme.*—It is generally believed that the folding of protein molecules is determined by their primary structures. Now that the structure of lysozyme is known, therefore, it is interesting to ask whether any indications of the folding process are revealed by a comparison of the three-dimensional and primary structures. It appears that there may be such indications.

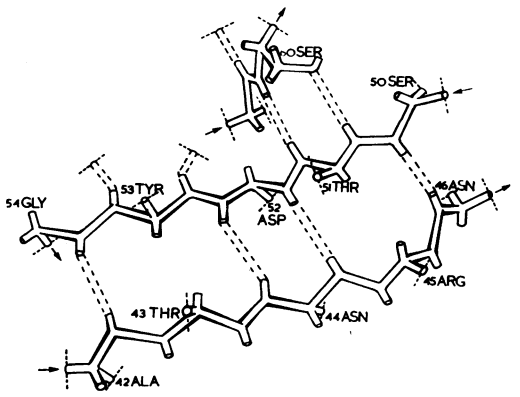


FIG. 3.—The region of antiparallel pleated sheet.

In view of the observed general distribution of hydrophilic and hydrophobic side chains in myoglobin and in lysozyme, it seems useful to consider the distribution of these properties throughout the primary structure. Following Dunnill and Phillips (to be published), this can be achieved by plotting the relative hydrophobicities of the amino acid residues as estimated by Tanford (1962) in the way shown in Figure 4. In this diagram the ordinates represent the free energy changes in cal/mole on transferring the free amino acids from ethanol to water at 25°C, and the abscissae are the residue numbers in the lysozyme sequence. Completely nonpolar side chains are marked by full circles, polar side chains by open circles, and ionizable side chains by triangles which point up for basic and down for acidic groups.

This representation of the amino acid sequence shows clearly that in hen egg-

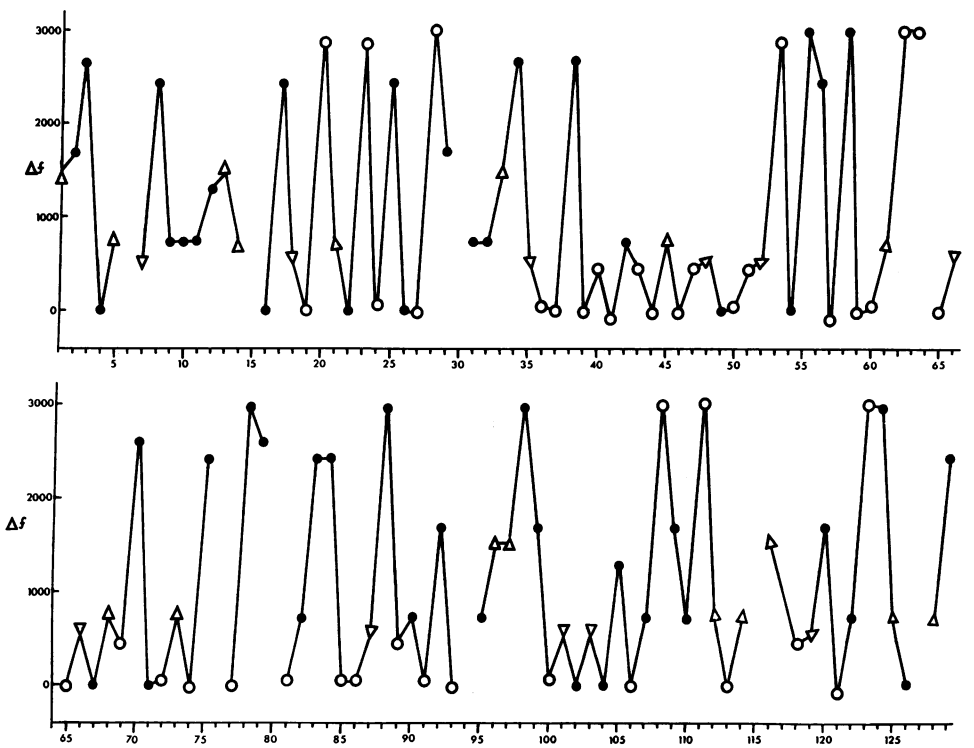


FIG. 4.—The variation of hydrophobicity in the primary structure of lysozyme. Ordinates cal/mole (see text).

white lysozyme the strongly hydrophobic residues are not distributed uniformly throughout the primary structure. In particular, residues 39–52 are all comparatively hydrophilic and comparison with the three-dimensional structure suggests that this is significant with respect to the mechanism of folding. Before this is considered further, however, it seems useful to note that protein molecules are synthesized from their terminal amino ends (Dintzis, 1961; Canfield and Anfinsen, 1963) and to suggest that the folding process may begin during synthesis and that some features of the conformation adopted by the nascent polypeptide chain may be recognizable in the completed molecule (Dunnill, 1965; Dunnill and Phillips, to be published).

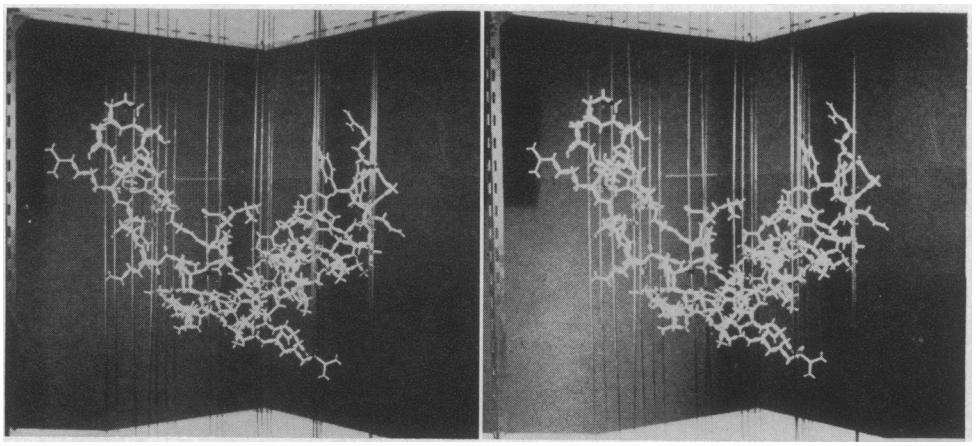
According to this view it is illuminating to consider the conformation of progressively larger sections of the protein molecule, starting at the terminal amino end, and Figure 5 illustrates the structure of lysozyme broken down in this way. It at least has the merit of facilitating a general description of the molecule.

The first 40 residues include a high proportion that favor α-helix formation (e.g., Guzzo, 1965; Prothero, 1966) and a number of hydrophobic side chains of which some are distributed so as to give helices with partially hydrophobic surfaces (cf. Perutz *et. al.*, 1965). In the finished molecule this part of the sequence forms a compact globular unit with hydrophobic side chains providing the contacts between two α-helices (right-hand wing of Fig. 5*a*). The following 12 residues, on the other hand, constitute the comparatively hydrophilic group mentioned above, and in the finished structure they form the antiparallel pleated sheet (left-hand wing of Fig. 5*a*). The obvious implication here is that in an aqueous environment there is no incentive for these residues to aggregate with the rest of the folded chain until the hydrophobic residues 55 and 56 require shielding, when the chain folds back on itself, forming the pleated sheet and burying these residues in the existing hydrophobic pocket.

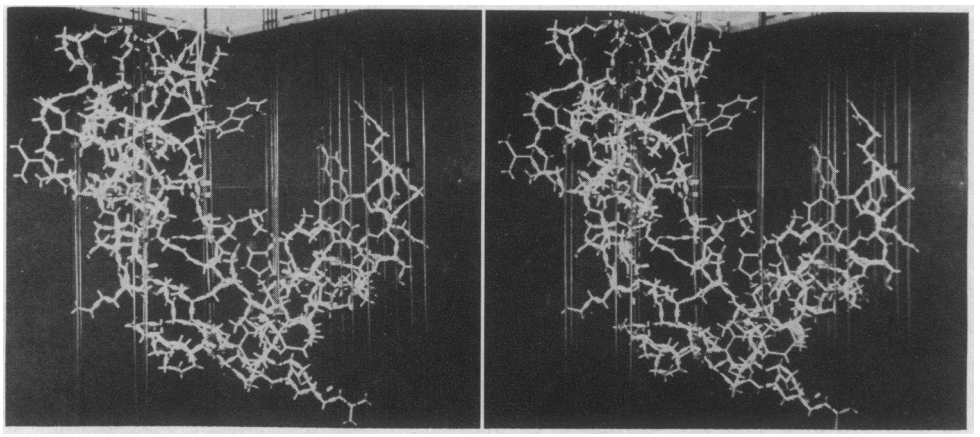
Residues 56–86 are folded rather irregularly in contact with the pleated sheet so that residues 1–86 (Fig. 5*b*) constitute a structure with two wings. Residues 88–100 form a rather irregular α-helix, again with a partially hydrophobic surface, which bridges the gap between these two wings, burying its hydrophobic side chains in the interior and leaving a groove down one surface of the molecule that is believed to be the site of catalytic activity (Fig. 5*c*). Finally the last 20 residues are folded around the globular unit built up by the first 40, so that it is clear at least that in this molecule the amino end of the chain must fold before the carboxyl end.

5. *The Activity of Lysozyme.*—The activity of lysozyme in promoting the dissolution of bacterial cell walls is now known to depend upon its ability to catalyze the hydrolysis of β-(1–4) glycosidic linkages between amino sugar residues in polysaccharide components of these cell walls. The evidence for this has been reviewed a number of times recently, notably at a discussion meeting of the Royal Society of London in February 1966, at which a preliminary account of our crystallographic studies of the activity of lysozyme was also given (Blake *et al.*, 1967). The cell-wall substrate is a copolymer in which alternate N-acetylglucosaminyl and N-acetylmuraminyl residues are joined by β-(1–4) glycosidic linkages and lysozyme catalyzes the hydrolysis of the linkage between N-acetylmuraminyl and N-acetylglucosaminyl residues as shown in Figure 6. Lysozyme also catalyzes the hydrolysis of chitin, the analogous polymer of N-acetylglucosamine, and many of

(*a*)



(*b*)



(*c*)

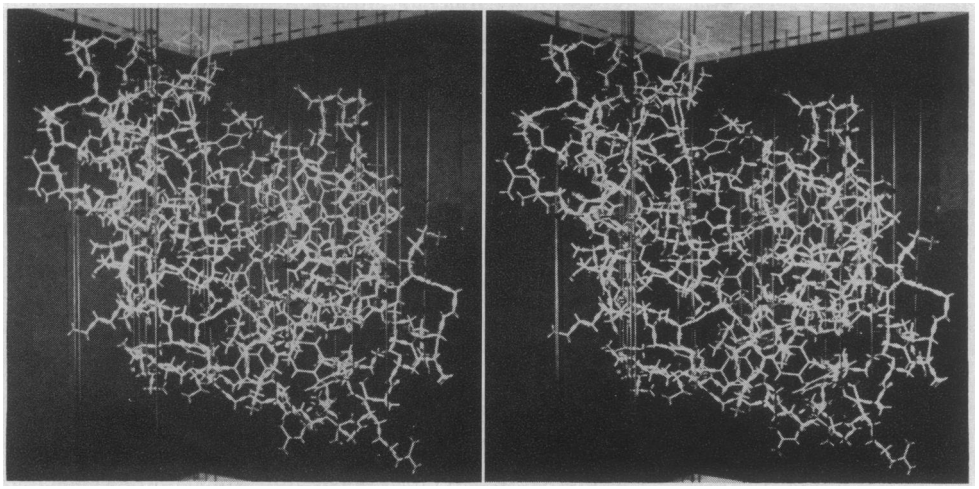


FIG. 5.—Stereo photographs of models of parts of the lysozyme molecule. (*a*) Residues 1–38; (*b*) residues 1–86; (*c*) residues 1–129 (complete molecule).

the recent studies of lysozyme have been concerned with its interaction with oligomers derived from chitin (e.g., Rupley, this meeting).

The starting point of our crystallographic studies was the observation by Wenzel, Lenk, and Schutte (1962) that the activity of lysozyme is inhibited competitively by N-acetylglucosamine. Following this lead we have investigated the binding of a number of amino sugars to lysozyme in the crystals, in the hope of being able to build up a picture of the enzyme/substrate complex. The technique is simply to diffuse the sugar molecules into preformed crystals, or to cocrystallize the enzyme with its inhibitor, and to locate the bound small molecule by direct calculation of the difference it produces in the electron density distribution. This involves remeasurement of the diffraction pattern and it is made possible, of course, by our knowledge of the phases of reflections from crystals of the enzyme alone.

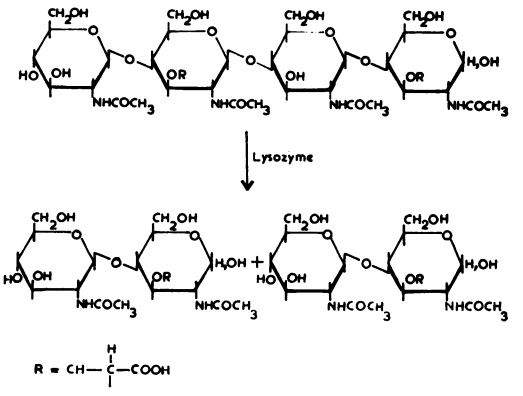


FIG. 6.—Hydrolysis of cell wall tetrasaccharide by lysozyme (Sharon, 1967).

In this way we have studied the binding of a number of inhibitors at 6 Å resolution (Johnson and Phillips, 1965; Johnson, 1965). They include (*a*) N-acetylglucosamine; (*b*) N-acetylmuramic acid; (*c*) 6-iodo-α-methyl-N-acetylglucosaminide; (*d*) α-benzyl-N-acetylmuramic acid; (*e*) di-N-acetylchitobiose; (*f*) N-acetylglucosaminyl-N-acetylmuramic acid; and (*g*) tri-N-acetyl-chitotriose. The results show that all of these molecules bind to lysozyme in or near the cleft in the enzyme surface that can be seen in Figure 5*c*. At this low resolution they appear to occupy a number of different sites lying in this cleft, but detailed interpretation had to await the results of studies at higher resolution.

The binding of N-acetylglucosamine: The binding of N-acetylglucosamine and of tri-N-acetylchitotriose has been studied at 2 Å resolution. At this resolution it can be seen that the binding of N-acetylglucosamine is complicated by the fact that as the result of mutarotation the molecule is present in solution in both the α- and β-forms. Both forms appear to bind to lysozyme in the crystals, though not at the same time to the same molecule, and the difference electron density map is a readily interpretable composite image of their binding. The most striking-looking interactions involved in this binding are very specific hydrogen bonds between the NH and the carbonyl oxygens of the acetamido side group on the sugar and the main chain CO and NH groups of amino acid residues 107 and 59, respectively. These interactions are essentially the same for N-acetylglucosamine molecules in both the α- and β-forms but the remaining interactions are different. β-N-acetylglucosamine molecules form hydrogen bonds between their O(6) and O(3) atoms and the NH groups of the tryptophan side chains 62 and 63, respectively, in the way shown for sugar residue *C* in Figure 7. α-N-acetyl-glucosamine, on the other hand, appears to be rotated roughly about an axis formed by the hydrogen bonds to the acetamido group so that the sugar ring lies lower down the cleft and a hydro-

gen bond can be made from O(1) to the main-chain NH of residue 109. In addition the binding of both types of molecule is stabilized by a number of nonpolar interactions which are less easy to list. It is important to note that the conformation of the enzyme molecule changes to some extent when the inhibitor is bound. Residue 62 moves by about 0.75 Å, in such a way as to narrow the cleft, and there are related small shifts particularly through the part of the molecule to the left of the cleft as seen in Figures 5 and 7.

The binding of tri-N-acetylchitotriose: At 2 Å resolution it is very clear that tri-*N*-acetylchitotriose forms a stable complex with lysozyme in the crystals. The trisaccharide is bound in the cleft with its free reducing group pointing downward as shown by residues *A*, *B*, and *C* in Figure 7 and with its terminal residue (*C*) making the same contacts with the enzyme as does β-N-acetylglucosamine. The second residue (*B*) makes many nonpolar contacts with the enzyme and there appear to be hydrogen bonds between its O(6) and Asp 101, between its ring oxygen O(5) and O(3)H of residue *C*, and between its O(3)H and O(5) of residue *A*. This hydrogen bonding between the sugar residues is that proposed by Carlström (1962) in his study of chitin. Residue *A* appears to be linked to the enzyme by nonpolar interactions and by a hydrogen bond between its NH group and the side chain of Asp 101. Again there are clear indications in the electron density map of a small conformational change in the enzyme when this inhibitor is bound. The shifts in atomic positions are closely similar to those observed on the binding of N-acetylglucosamine.

On the location of the catalytic site: It is most probable that the structure of the tri-N-acetylchitriose/lysozyme complex seen in the crystals is not that of a productive enzyme/substrate complex. The chemical evidence, which is reviewed by Professor Rupley in the following paper, is that the hydrolysis of tri-N-acetylchitotriose proceeds only slowly at the concentrations used in preparation of the crystals, probably because the preferred binding is unreactive, and it has been shown by repeated measurements of the diffraction pattern that the observed complex is quite stable in the crystals. It is reasonable to suppose, however, that the observed binding may represent a part of the true enzyme substrate complex that does not embrace the catalytic site and to wonder whether careful model building, based upon the structure of this complex, might reveal the site and mechanism of catalysis.

Model building shows that a fourth sugar residue *D*, added at the free reducing-group end of the trisaccharide, makes reasonable contacts with the atoms of the enzyme molecule except that its C(6) and O(6) atoms tend to make too close contacts with the main chain CO of residue 52, with Trp 108, and with the acetamido group of sugar residue *C*. This overcrowding can be relieved, however, by distortion of the normal chair conformation of residue *D* toward a conformation with C(6) in an axial position, as shown in Figure 7. Hydrogen bonding between O(6) and either CO(52) or Glu(35) then is possible. Additional residues *E* and *F* can be added without difficulty and a number of good interactions with the enzyme molecule suggest themselves. Satisfactory nonpolar contacts and an almost complete scheme of hydrogen bonding are indicated in Figure 7, but it is not suggested that this is the only arrangement that is possible.

If the tri-N-acetylglucosamine binding extended in this way by model building

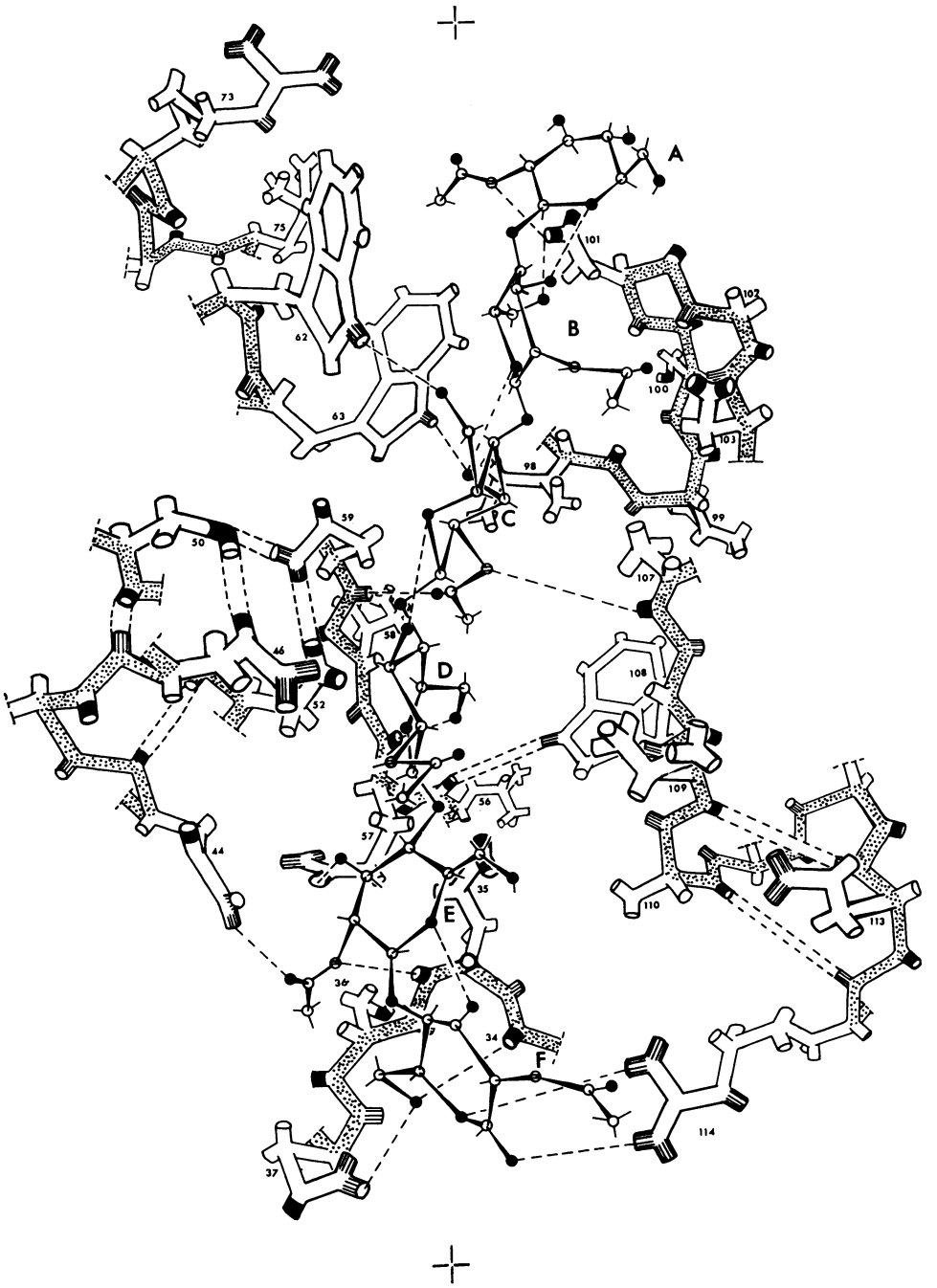


FIG. 7.—Atomic arrangement in the lysozyme molecule in the neighborhood of the cleft with a hexa-N-acetylchitohexaose shown bound to the enzyme. The main polypeptide chain is shown speckled and NH and O atoms are indicated by line and full shading, respectively. Sugar residues *A*, *B*, and *C* are as observed in the binding of tri-N acetylchitotriose (and β-N-acetylglucosamine for residue *C*). Residues *D*, *E*, and *F* occupy positions inferred from model building. It is suggested that the linkage hydrolyzed by the action of the enzyme is between residues *D* and *E*.

does show how the chitin substrate interacts with the enzyme and if the cell-wall substrate interacts with the enzyme in essentially the same way as chitin, then a further step towards identification of the catalytic site is possible. It is clear that *C* cannot be an N-acetylmuraminyl residue since its O(3)H group is directed into the cleft, where it is involved in hydrogen bonding, and there is no room for a lactyl group. In the binding of the cell-wall copolymer, therefore, only *B*, *D*, and *F* can be N-acetylmuraminyl residues. It follows from the above-mentioned assumptions that the glycosidic linkage affected by lysozyme must be between residues *B* and *C* or between *D* and *E*. Since residues *B* and *C* form part of the stable trisaccharide complex with the enzyme, however, it is reasonable to suggest that lysozyme promotes hydrolysis of the glycosidic linkage between residues *D* and *E*. Happily there is immediate support for this in the finding of Rupley (following paper) that hexa-N-acetylchitohexaose is hydrolyzed by lysozyme to form a tetramer and a dimer, the cleavage separating two residues from the reducing end of the oligomer.

The most reactive-looking groups in this region of the enzyme are provided by residues Glu 35 and Asp 52, the side chains of which are disposed on either side of the β-(1–4) linkage in question. These side chains have markedly different environments. The side chain of Glu 35 lies in a predominantly nonpolar region near the bottom of the cleft, while Asp 52, on the other hand, lies in an essentially polar region on the side of the cleft and appears to be involved in a network of hydrogen bonds that involves residues Asn 46, Ser 50, and Asn 59. (The identification of residue 59 is one of the points of disagreement between Canfield and Jollès (Blake *et al.*, 1967). The indications in the electron density map support Canfield's sequence Ile (58), Asn (59), but Jollès favors Asn (58), Ile (59). It is clearly important that the disagreement over this important part of the molecule should be resolved as soon as possible.) It is tempting to suppose that these environments may tend to hold Asp 52 in the ionized carboxylate state, while Glu 35 remains protonated at relatively high pH values.

In the model the distance between the glycosidic oxygen-linking residues *D* and *E* and the nearest oxygen of Glu 35 is almost 3 Å. On the other side the nearest oxygen of Asp 52 is about 3 Å from the C(1) atom of sugar residue *D* and about the same distance from the ring oxygen atom of that residue. These distances are subject, of course, to the errors involved in deriving atomic positions from the electron density map, and they are also dependent upon how the substrate model is positioned. It must be remarked that, although only small adjustments to the position and orientation of the substrate model appear to be possible, it is not claimed at this stage that all other orientations, positions, and conformations of the mucopolysaccharide have been completely ruled out. The arrangement suggested here is, however, the only one found so far to be sterically satisfactory.

The chemical evidence (Rupley, following paper) is that the bond broken is that between C(1) and the glycosidic oxygen, and it is interesting to consider how this reaction might be promoted by the environment shown in the model. In fact, thanks in part to discussions with Dr. C. A. Vernon, a plausible mechanism can be proposed. It is suggested that the reaction is promoted by the concerted action of residues 52 and 35 in which Glu 35 donates a proton to the glycosidic oxygen, while the negatively charged Asp 52 stabilizes an intermediate carbonium ion at C(1) of sugar residue *D*. Lemieux and Huber (1955) have suggested that the

charge on such a carbonium ion will be shared with the ring oxygen atom and that consequently the sugar ring will take up the half-chain conformation. But this is the very conformation which seems to be required to allow binding of sugar residue *D* to the enzyme, and it appears, therefore, that this distortion of the substrate also plays a part in the catalysis. The enzyme apparently binds the reaction intermediate, an aspect of the action of enzymes which was discussed by W. P. Jencks (Brandeis University) in the Symposium on Future Directions in Polymer Chemistry at this meeting and which is probably of general importance.

This proposed scheme of catalysis clearly is open to various experimental tests and can be investigated further in crystallographic studies of the kind described here. As the following paper by J. A. Rupley will show, there is already a considerable body of experimental evidence which can be considered in the light of the model and used to test it.

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